

## *Seychellomyces sinensis* sp. nov. from China

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**ABSTRACT** —An aquatic hyphomycete, *Seychellomyces sinensis*, isolated from China, is proposed as a new species. Phylogenetic analysis of combined ITS, LSU, 60S, and MCM7 sequences place *Seychellomyces* in *Ceratocystidaceae*. The new species, characterized by septate conidia and elliptical to cylindrical (sub)hyaline endoconidia, is distinguished from *S. hexagonus* by its larger, 1–3-septate conidia.

**KEY WORDS**—asexual fungi, *Microascales*, *Sordariomycetes*, taxonomy

## Introduction

Aquatic hyphomycetes are fungi that most commonly occur on dead leaves in streams (Bärlocher 1992). These fungi are a polyphyletic group that play a key role in processing organic matter in freshwater (Belliveau & Bärlocher 2005). Traditionally, species have been identified through their conidial morphology, but phylogenetic placements of many species have not yet been determined. The introduction of molecular analysis to fungal taxonomy has recently verified the phylogenetic placement of some species (Duarte & al. 2015).

*Seychellomyces* Matsush. was proposed for a single species, *S. hexagonus* Matsush. (Matsushima 1981) and remains monotypic. It is characterized by

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producing septate brown to pale brown conidia that are hexagonal in cross-section and aseptate hyaline endoconidia (Matsushima 1981, Seifert & al. 2011).

Several new hyphomycetes species have been reported from southwest China (Qiao & al. 2017a,b; 2018a,b, 2019; Yu & al. 2019). During further study of the aquatic hyphomycetes, a specimen with two types of conidia was found growing on submerged dicotyledonous leaves. The specimen was quite close to *Seychellomyces hexagonus* in its conidiogenous features but differed in possessing larger and more septate conidia. Here we describe it as a new species and present the phylogenetic position of *Seychellomyces* as determined by combined sequences of internal transcribed spacer (ITS), large subunit nuclear ribosomal RNA (LSU), 60S ribosomal protein gene RPL10 (60S), and the minichromosome maintenance complex component 7 (MCM7).

## Materials & methods

### Collection, fungal isolation, and morphological characterization

Submerged dicotyledonous leaves were collected from a stream in Sangsi, Guangxi Province, China. Samples were preserved in zip-lock plastic bags, labelled, and transported to the laboratory. Each rotted leaf was cut into several 3–4 × 4–5 cm fragments that were incubated on CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) for 5 days at room temperature. Individual conidia were isolated using a sterilized toothpick under a BX51 microscope and cultivated on CMA plates. Morphological observations were conducted on cultures growing on CMA, V-8 juice agar (200 ml Campbell V-8 juice, 3.0 g CaCO<sub>3</sub>, 18 g agar, 800 ml distilled H<sub>2</sub>O), and PDA (200 g potato, 20 g dextrose, 18 g agar, 1000 ml distilled H<sub>2</sub>O) after incubation at 25°C for one week.

Pure cultures were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF; formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan).

### DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fresh mycelia grown on potato dextrose agar (PDA) at 25°C as described by Turner & al. (1997). Four gene regions were amplified: ITS with primer pair ITS5/ITS4 (White & al. 1990); LSU with LROR/LR7 (Vilgalys & Hester 1990); 60S ribosomal protein RPL10 (60S) with 60S-506F/60S-908R (Nel & al. 2018); and MCM7 with MCM7-for/MCM7-rev (Nel & al. 2018). The PCR thermal cycle programs for the amplifications of these four DNA fragments followed those described in de Beer & al. (2014). PCR products were then purified

TABLE 1. Strains and GenBank accession numbers of sequences used in molecular phylogenetic analyses

| SPECIES                            | STRAIN     | GENBANK NUMBER |          |          |          |
|------------------------------------|------------|----------------|----------|----------|----------|
|                                    |            | ITS            | LSU      | 6oS      | MCM7     |
| <i>Ambrosiella xylebori</i>        | CMW110.61  | NR_144921      | KM495318 | KM495495 | KM495407 |
| <i>Berkeleyomyces basicola</i>     | CMW6714    | MF952423       | MF948658 | MF967072 | MF967079 |
|                                    | CMW49351   | MF952428       | MF948659 | MF967075 | MF967102 |
|                                    | CMW25439   | MF952427       | —        | —        | MF967099 |
| <i>Berkeleyomyces rouxiae</i>      | CMW5472    | MF952406       | MF948657 | MF967074 | MF967080 |
|                                    | CMW14219   | MF952402       | MF948660 | MF967076 | MF967086 |
| <i>Ceratocystis diversiconidia</i> | CMW22445   | FJ151440       | KM495334 | KM495511 | KM495423 |
| <i>Ceratocystis eucalypticola</i>  | CMW11537   | FJ236723       | KM495339 | KM495516 | KM495428 |
| <i>Ceratocystis fimbriata</i>      | CMW15049   | AY953378       | KM495343 | KM495520 | KM495432 |
| <i>Ceratocystis pirilliformis</i>  | CMW6579    | AF427105       | KM495365 | KM495542 | KM495453 |
| <i>Ceratocystis platani</i>        | CMW14802   | DQ520630       | KM495366 | KM495543 | KM495454 |
| <i>Chalaropsis ovoidea</i>         | CMW22733   | FJ411343       | KM495400 | KM495577 | KM495487 |
| <i>Chalaropsis thielavioides</i>   | CMW22736   | FJ411342       | KM495402 | KM495579 | KM495489 |
| <i>Davidsoniella australis</i>     | CMW2333    | FJ411325       | KM495396 | KM495573 | KM495483 |
| <i>Davidsoniella eucalypti</i>     | CMW3254    | FJ411327       | KM495338 | KM495515 | KM495427 |
| <i>Endoconidiophora coerulea</i>   | CMW26365   | FJ411322       | KM495329 | KM495506 | KM495418 |
| <i>Endoconidiophora douglasii</i>  | CMW26367   | NR_120295      | KM495335 | KM495512 | KM495424 |
| <i>Graphium pseudormiticum</i>     | CMW503     | AY148186       | KM495390 | KM495567 | KM495477 |
| <i>Huntia chinaeensis</i>          | CMW24658   | JQ862729       | KM495327 | KM495504 | KM495416 |
| <i>Huntia moniliformis</i>         | CMW10134   | FJ151422       | KM495355 | KM495532 | KM495443 |
| <i>Huntia oblonga</i>              | CMW23803   | EU245019       | KM495359 | KM495536 | KM495447 |
| <i>Seychellomyces sinensis</i>     | YMF1.04178 | KU549178       | MG830704 | MK554714 | MK554715 |
| <i>Thielaviopsis cerberus</i>      | CMW36668   | NR_145225      | KM495326 | KM495503 | KM495415 |
| <i>Thielaviopsis musarum</i>       | CMW1546    | JX518325       | KM495357 | KM495534 | KM495445 |
| <i>Thielaviopsis paradoxa</i>      | CMW36689   | JX518342       | KM495363 | KM495540 | KM495451 |
| <i>Thielaviopsis radicola</i>      | CMW1032    | KF612023       | KM495371 | KM495548 | KM495459 |

using a Biotek DNA Gel/PCR Purification Kit and forward and reverse sequenced with a LI-COR 4000L automatic sequencer, using a Thermo Sequenase-kit as described by Kindermann & al. (1998).

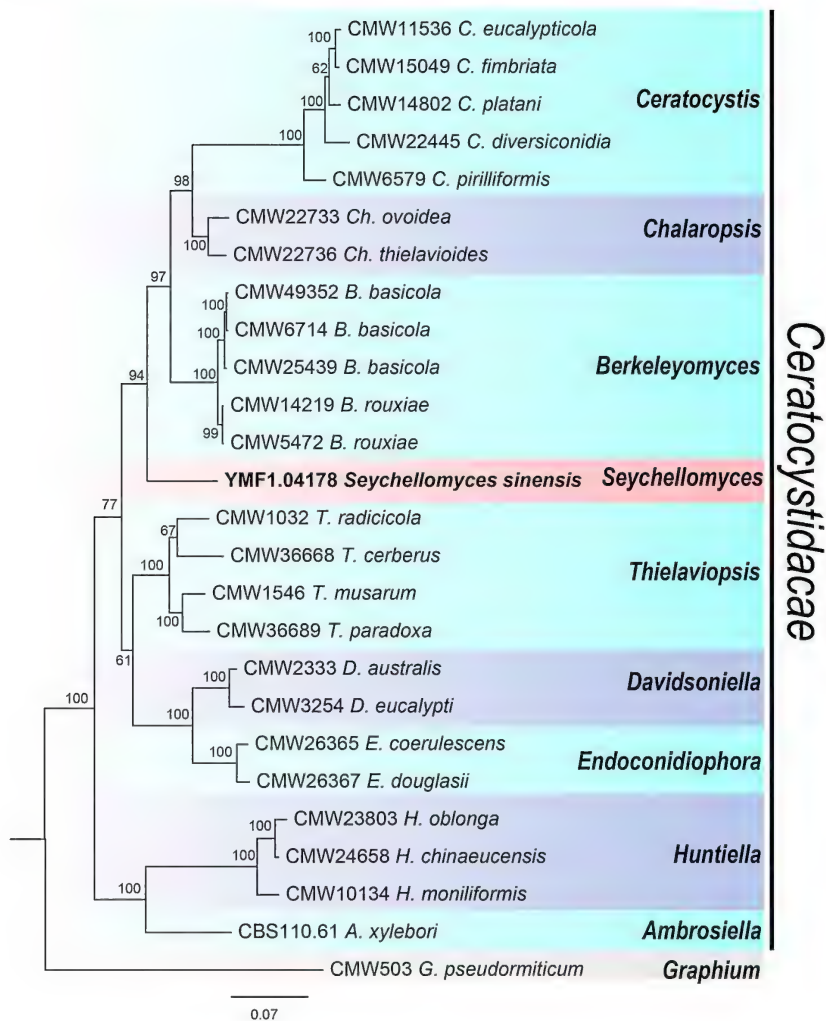


PLATE 1. MrBayes tree generated from a combined alignment of ITS, LSU, 60S, and MCM7 sequences of *Ceratocystidaceae*, with *Graphium pseudodormiticum* (*Microasceae*) as outgroup.

### Sequence alignment and phylogenetic analysis

The sequence dataset contained the ITS, LSU, 60S and MCM7 sequence data for 21 species representing eight genera in the *Ceratocystidaceae* (*Microascales*). *Graphium pseudodormiticum* M. Mouton & M.J. Wingf. (*Microasceae*) was chosen as outgroup based on Nel & al.'s (2018) phylogenetic study, and the sequence data were downloaded from the NCBI GenBank database (TABLE 1).

DNA sequence data were aligned using ClustalX 1.83 (Higgins 1994) with default parameters, and the consensus sequences were adjusted manually and linked in BioEdit v.7.0 (Hall 1999). MrBayes (Ronquist & Huelsenbeck 2003) was used to calculate the concatenated sequence-based tree, with following parameters: ngen = 1,000,000; samplefr = 1000; printfr = 1000.

### Phylogenetic results

The final alignment included a total of 2022 base pairs. The phylogenetic tree was generated by combining the ITS, LSU, 60S, and MCM7 sequences and using Bayesian analysis. The topology of the tree is shown with the bootstrap support value for each node (PLATE 1). The tree supports *Seychellomyces* within the *Ceratocystidaceae*; the genus does not group with any other genera but is associated with the lineage (94% support) containing *Berkeleyomyces*, *Chalaropsis*, and *Ceratocystis* in a basal position.

### Taxonomy

*Seychellomyces sinensis* Z.F. Yu & Hua Zheng,

PLATE 2

MB 829084

Differs from *Seychellomyces hexagonus* by its larger 1–3-septate conidia.

TYPE: China, Guangxi province, Shangsi, Shiwan Mountain, 21°34'49"N 109°14'20"E, alt. 23 m, on decaying leaf of an unidentified broadleaf tree, 7 July 2013, Z.F. Yu. (Holotype, YMFT 1.04178 [permanent slide]; ex-type living culture, YMF 1.04178; GenBank KU549178, MG830704, MK554714, MK554715).

ETYMOLOGY: From the Latin, *sinensis*, for the country of origin, China.

COLONIES attaining 3 cm diam. after 7 days on CMA, effuse, pale brown to brown. Mycelium sparse, hyphae branched, septate, hyaline or pale brown. CONIDIOPHORES arising from the creeping hyphae, cylindrical, 2(–5)-septate, erect, unbranched, smooth, 12–40 × 5–7 µm (narrowing to 3.5–5 µm at the apex). CONIDIOGENOUS CELLS monoblastic, integrated, terminal, determinate. CONIDIA ellipsoidal, brown to pale brown, smooth or sometimes with a warty surface, growing singly or 2–3 in chains, 1–3-septate (mainly 2-septate), with short (4–13 µm long) cylindrical conidiophore remnants at both ends, in cross-section hexagonal (occasionally pentagonal or quadrangular). ENDOCONIDIA originating endogenously within phialides, catenulate, variable in length, hyaline or subhyaline, elliptical to cylindrical, truncate at ends, occasionally repetitiously germinating by producing a short conidiophore from which a phialide is formed to produce a chain of endoconidia. CHLAMYDOSPORES ochraceous, globose, smooth, 5–10 µm in diam.

CONIDIAL SIZES ON DIFFERENT MEDIA—ON V-8 JUICE AGAR: 1-septate,  $20\text{--}23 \times 12\text{--}16\text{ }\mu\text{m}$ , total length (including short cylindrical conidiophore remnants)  $34\text{--}39\text{ }\mu\text{m}$ ; 2-septate,  $29\text{--}39 \times 16\text{--}21\text{ }\mu\text{m}$ , total length  $40\text{--}56\text{ }\mu\text{m}$ ; 3-septate  $35\text{--}49 \times 11\text{--}15\text{ }\mu\text{m}$ , total length  $55\text{--}35\text{ }\mu\text{m}$ ; endoconidia  $5\text{--}21 \times 3\text{--}5.7\text{ }\mu\text{m}$ .

ON PDA: 1-septate,  $20\text{--}27 \times 13\text{--}21\text{ }\mu\text{m}$ , total length  $31\text{--}45\text{ }\mu\text{m}$ ; 2-septate,  $38\text{--}50 \times 19\text{--}30\text{ }\mu\text{m}$ , total length  $42\text{--}69\text{ }\mu\text{m}$ ; 3-septate  $38\text{--}56 \times 19\text{--}27\text{ }\mu\text{m}$ , total length  $53\text{--}73\text{ }\mu\text{m}$ ; endoconidia  $7\text{--}39 \times 3\text{--}5\text{ }\mu\text{m}$ .

ON CMA: 1-septate,  $23\text{--}30 \times 15\text{--}21\text{ }\mu\text{m}$ , total length  $34\text{--}47\text{ }\mu\text{m}$ ; 2-septate,  $31\text{--}51 \times 19\text{--}31\text{ }\mu\text{m}$ , total length  $42\text{--}70\text{ }\mu\text{m}$ ; 3-septate,  $42 \times 20\text{ }\mu\text{m}$ , total length  $58\text{ }\mu\text{m}$ ; endoconidia  $6\text{--}42 \times 3\text{--}5\text{ }\mu\text{m}$ .

## Discussion

*Seychellomyces* has been treated as monotypic since the genus was established in 1981. Based on characteristic of conidia and endoconidia, our strain was easily identified to *Seychellomyces*. However, *S. sinensis* is distinguished from the type species, *S. hexagonus*, by having 1–3-septate conidia, while conidia of *S. hexagonus* are always 2-septate. In addition, conidia of *S. hexagonus* are always hexagonal in cross-section (Matsushima 1981), whereas those of *S. sinensis* are mostly hexagonal, but occasionally pentagonal or quadrangular. The size of conidia of *S. sinensis* differs based on growth medium and conidial septation; but on V-8 juice agar medium the 2-septate conidia of *S. sinensis* are larger than those of *S. hexagonus* ( $25\text{--}33 \times 14\text{--}18\text{ }\mu\text{m}$ ; Matsushima 1981). Globose chlamydospores were observed in culture of *S. sinensis*, but not mentioned in *S. hexagonus*.

Among *Ceratocystidaceae*, only *Thielaviopsis* sensu lato has a superficial resemblance to *Seychellomyces* in conidial shape (Seifert & al. 2011). However, *Thielaviopsis*, *Chalaropsis*, and *Ceratocystis* significantly differ from *S. sinensis* phylogenetically.

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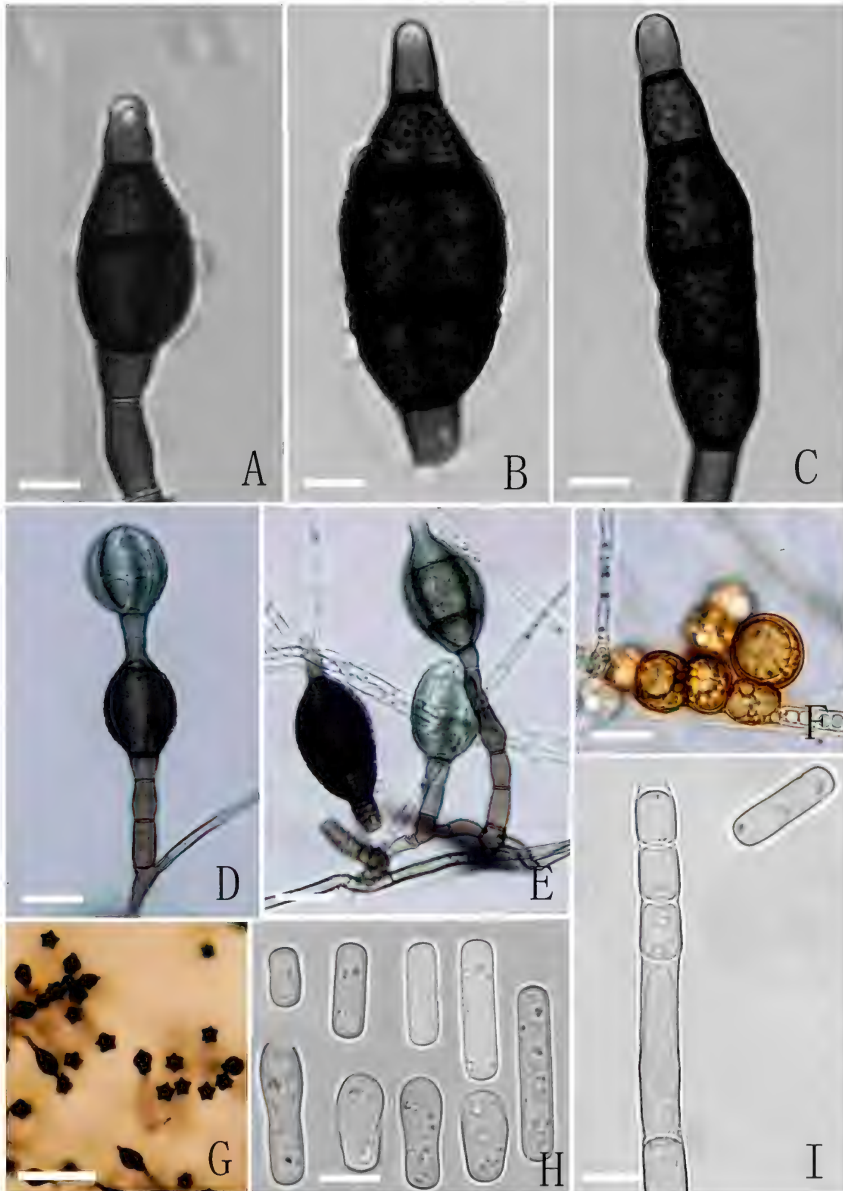


PLATE 2. *Seychellomyces sinensis* (holotype, YMF 1.04178): A–C. Conidia; D, E. Conidia with conidiophores; F. Chlamydospores; G. Cross-section of conidia. H. Endoconidia. I. Conidiogenous cells of endoconidia. Scale bars: A–C, F, H, I = 10  $\mu$ m; D, E = 20  $\mu$ m; G = 100  $\mu$ m.

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